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EFFECTS OF BIOTIN ON FATTY LIVER DISORDERS IN
POULTRY

BY

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ABSTRACT

Rations containing different levels of protein and biotin were fed to broiler chicks and turkey poults to study the effects on rate of growth, symptoms of deficiency and the occurrence of fatty liver and kidney syndrome (FLKS). A study was also conducted to assess the incidence of haemorrhagic liver syndrome (HLS) in laying hens fed rations containing different levels of Span rapeseed meal and biotin. The livers and kidneys of the broiler chickens and the livers of the hens were analyzed for moisture, fat and protein.

The addition of biotin to broiler rations containing a high or medium level of protein resulted in a more rapid rate of growth and reduced the amount of feed required per unit of gain. A lower incidence of perosis was also observed in the chicks fed rations supplemented with biotin. No symptoms of FLKS were seen in any of the chicks. The composition of the livers and kidneys was not affected by levels of protein in the ration or the amount of biotin fed, but a reduction in liver size was observed when biotin was added to the rations.

The addition of biotin to turkey starting rations of varying protein content increased the rate of growth of poults as compared to those fed unsupplemented rations.

Dermatitis that occurred on the unsupplemented rations was eliminated and incidence of perosis was reduced when biotin was added to the rations. No symptoms of FLKS were observed in poults fed any of the rations. The composition of the livers and kidneys of the poults was not affected by the addition of biotin to the rations fed. A reduction in fat and protein content of the liver of poults fed a low protein ration was observed.

Use of a low level (5%) of Span rapeseed meal in laying rations had no effect on mortality, rate of production or egg quality; however, inclusion of 10 or 15% of the meal increased mortality and reduced egg production and Haugh unit values. Mortality due to haemorrhagic liver syndrome increased when 10 or 15% rapeseed meal was included in the ration, but no mortality from haemorrhagic liver syndrome (HLS) was observed on rations containing 0 or 5% of rapeseed meal. The use of biotin in the rations had no effect on level of mortality or mortality caused by HLS. The composition of livers and kidneys of laying hens was not significantly affected by either inclusion of biotin in the rations or level of rapeseed meal fed.

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INTRODUCTION

A disorder known as fatty liver and kidney syndrome (FLKS) has been responsible for heavy losses in broiler flocks. The level of mortality encountered has ranged from 1 or 2% to levels as high as 30%. As a consequence there has been much concern within the poultry industry respecting incidence and control of this disorder.

As the name of the disorder implies, one of the principal symptoms of the syndrome consists of increased deposition of fat in the liver and kidneys of broilers. Affected birds are lethargic and death occurs within a few hours. Mortality is usually seen in birds 18 to 23 days of age but may occur at any time during the growing period.

Research conducted on this problem has indicated that outbreaks of the syndrome often occurred on diets containing high levels of wheat or maize and suboptimal levels of protein. Recently it was shown that biotin, one of the B-vitamins, was effective in preventing the occurrence of the disorder.

A somewhat similar disorder has also been reported in laying hens. This disorder has been referred to as fatty liver syndrome, fatty liver haemorrhagic syndrome or haemorrhagic liver syndrome (HLS). In this disorder the liver is pale in color and friable suggesting an increase in

fat content. Mortality that occurs is caused by massive haemorrhages in the liver. There are no reports indicating that the kidneys are involved in the appearance of the disorder in laying hens.

There has been no indication that the addition of biotin to laying hen's rations is effective in reducing the incidence of HLS; nevertheless the occurrence of HLS may be affected by the composition of the diet. Recent studies have indicated that the use of rapeseed meal (RSM) in laying rations at levels in excess of that usually recommended (5%) resulted in an increase in the incidence of HLS.

Since several factors may be involved in the occurrence of FLKS and HLS, and although the two conditions may be entirely unrelated, it seemed desirable to study the disorders in more detail. Consequently, experiments were initiated to study the effects of use of biotin on the incidence of FLKS and levels of fat in the livers and kidneys of broilers and turkeys poultts fed rations of varying protein content. In addition a study was conducted to assess the effects of use of biotin and RSM on the incidence of HLS in laying hens. Levels of fat in the livers and kidneys of laying hens were also determined.

LITERATURE REVIEW

A. Fatty liver and kidney syndrome in broilers

i. Symptoms of the disorder:

Fatty liver and kidney syndrome was first described by the Danish workers Marthedal and Velling (1958) cited by Hemsley (1965). They described the disease as one of unknown aetiology, the typical feature of which is an increase in the amount of fat deposited in the liver, kidney and myocardium. Since the disease seemed to affect only chicks from certain farms, it was suggested that outbreaks might be a result of interaction between factors carried by the chick and environmental factors. No suggestion of dietary involvement was made because the disorder was seen in chicks fed six commercial brands of feed.

FLKS is a disorder which affects young chicks, with mortality usually occurring between the 18th and 23rd day of age. (Hemsley, 1965; Blair, Bolton and Duff, 1969). In most instances, losses are relatively low (1-2% mortality) but occasionally outbreaks occur with much higher mortality levels (Whitehead and Blair, 1974; Payne et al., 1974). The external symptoms of the disorder are not specific for FLKS alone. Affected birds often appear lethargic. Some birds may be found lying with their heads bent over their backs. When this stage is reached death usually occurs within a few hours (Blair and Whitehead, 1974).

The internal symptoms seen in FLKS serve as a more reliable guide for identifying the disease. On examination of affected birds, it has been noted that the livers and kidneys were enlarged and pale. The color of the liver may range from a brown to greyish-white color, with haemorrhages on either one or both lobes ranging from pin-point size to large areas. Sometimes the haemorrhages were seen around the edge of the liver. The kidneys of affected birds were also swollen and pale. Lesions were observed in kidneys at the proximal and distal convoluted tubules and the tubules were swollen to such an extent that other renal structures, especially the glomeruli and distal convoluted tubules, were so crowded together that they were compressed (Hemsley, 1965; Marthedal, Velling and Jylling, 1974). In the kidney the mitochondria and other organelles of the cell were not degenerated but numerous large globules of lipid were present in the cytoplasm. The presence of large granular globular lipid bodies both inside and outside of liver cells have also been reported (Whitehead et al., 1973) cited by Blair and Whitehead (1974). Some degeneration in heart muscle fibres has been mentioned together with sporadic occurrence of fatty droplets (Mathedal, Velling and Jylling, 1974). Gross changes in the adrenals of birds affected with FLKS were observed by Payne et al., (1974). Changes in the gizzard and duodenum have also been noted (Hemsley, 1965;

Blair and Whitehead, 1974).

Studies on the lipid metabolism of chicks affected with FLKS have also shown that changes occur. The levels of triglycerides in the liver and kidneys were increased as much as 4 to 5 times those observed in normal birds. (Whitehead, 1975; Johnson et al., 1972 cited by Whitehead, 1975). The higher levels of triglycerides were accompanied by increased palmitoleic acid and decreased stearic acid contents of the liver and kidneys (Whitehead, 1975). The levels of phospholipids and the fatty acid composition of adipose tissue were not markedly altered (Evans, Bannister and Whitehead, 1975).

Changes in plasma free fatty acid concentration of chicks affected with FLKS were reported by Evans, Bannister and Whitehead (1975). They found that the concentration of free fatty acids in the plasma was invariably increased. This was probably due to increased mobilization of lipid depots rather than to a reduction in uptake or utilization of fatty acids since fatty acid oxidation in the liver, kidney and heart was normal.

ii. Factors affecting the incidence of FLKS

The first suggestion that nutritional factors might be of importance in the development of FLKS was made by Blair, Bolton and Duff, (1969). They observed that the level of

mortality from FLKS appeared to be inversely related to the protein level of the diet. It was suggested that the sporadic appearance of the disorder might be due to an interaction of genetic, environmental and dietary factors.

Blair, Bolton and Duff (1969) and Laursen-Jones (1971) cited by Blair, Whitehead and Teague (1975) also observed the effect of low protein level on mortality from FLKS and indicated that the disorder was most likely to occur on diets containing a high level of wheat. In later trials, however, it was noted that when wheat was replaced isocalorically by barley and starch, the level of mortality was as high (14%) as on the ration containing wheat. The non-specific effect of source of grain on the occurrence of the disorder has been observed by others (Blair et al., 1973; Blair, Whitehead and Teague, 1975).

There is considerable evidence that incidence of FLKS may be affected by levels of fat in the diet. Husbands and Laursen-Jones (1969) observed that mortality from FLKS was nil on diets containing 4% maize oil or 4% lard and 18.4% crude protein, as compared to FLKS levels ranging from 10 to 19% on similar rations of lower protein content without added fat. Blair et al. (1973) also observed that mortality from FLKS was reduced when the dietary fat level was increased. It was suggested that FLKS may be related to a disorder of fat metabolism. In a subsequent report Blair,

Whitehead and Teague (1975) observed that high levels of protein and fat in the diet had a protective influence on mortality from FLKS. Whitehead et al. (1975) reported that the addition of maize oil, tallow or olive oil, isoenergetically at the expense of starch in the diet, reduced mortality due to FLKS from 19 to 7 per cent.

Several other factors have also been implicated in the occurrence of FLKS in broilers. Diets of high energy to protein ratio based on wheat or barley have been found to consistently cause high mortality from FLKS. (Whitehead and Blair, 1974; Blair, Whitehead and Teague, 1975). In addition environmental factors such as high temperature or any other kind of stress have been shown to affect the incidence of this syndrome. (Whitehead and Blair, 1974; Whitehead et al., 1975). Sex influence may also be involved. Blair, Whitehead and Teague (1975) and Whitehead et al. (1975) observed a higher incidence of mortality due to FLKS among females than males. The possibility that genetic factors may affect the incidence of mortality due to FLKS to some degree was mentioned by Hemsley (1965) and Blair, Bolton and Duff (1969). Blair, Whitehead and Teague (1975) found that mortality was increased on a pelleted diet as compared with a mash diet. This suggests the possibility that one or more nutrients might be destroyed or modified by the pelleting process.

The possibility that a vitamin or some other nutrient might be involved was indicated by the report of Hemsley (1973). They observed that the addition of molasses to the drinking water was effective in greatly reducing mortality from FLKS.

Following upon the observations of Hemsley (1973), it has been shown that the addition of biotin to the rations fed was effective in preventing losses from FLKS. Payne et al. (1974), reported that mortality from FLKS could be stopped by administering yeast and ascorbic acid to broilers. Subsequently it was observed that a liquid vitamin mixture containing biotin was effective in controlling mortality from FLKS under experimental conditions. Under field conditions, administration of a liquid vitamin mix containing biotin also halted mortality from FLKS on a diet composed mainly of wheat, wheat by-products, and meat and bone meal. Since a similar vitamin mixture without added biotin had been used previously without effect on FLKS, this suggested that diets high in wheat and barley, in which the non-cereal protein was not predominantly soybean meal may require supplemental biotin.

Blair and Whitehead (1974) also observed that the addition of a vitamin supplement containing most of the vitamins, including biotin, reduced mortality from 21% to

1%. In another trial when 24 diets were supplemented with individual vitamins or combinations of the vitamins, it was observed that mean mortality from FLKS was 0.2% with diets containing added biotin whereas mortality was 23% on the diets without biotin. Whitehead, Bannister and Wight (1974) also reported that mortality was reduced to zero by adding biotin to low and high protein diets which when not supplemented with biotin consistently caused 20 and 4% FLKS mortality respectively. On the basis of the above work it was concluded that FLKS is a biotin responsive syndrome even though other symptoms of biotin deficiency were not usually seen.

B: Haemorrhagic liver syndrome

i: Symptoms of haemorrhagic liver syndrome

A disorder somewhat similar to FLKS has been observed in laying hens. This condition was first reported by Couch (1956) who called it "Fatty liver syndrome".

The disorder which usually occurred in the second half of the laying year, was characterized by a decrease in rate of egg production and increased mortality. When the livers of the hens that died were examined they were found to be greatly enlarged with a uniform light-yellow color and friable consistency. Haemorrhages and haematomas were also noted in the livers. Affected birds usually showed extensive accumulation of abdominal fat. Death of the birds

was caused by massive haemorrhages which resulted in rupture of the Glisson's capsule (Couch, 1956; Price et al., 1957; Nesheim, Ivy and Norvell, 1969; Ivy and Nesheim, 1973; Jensen et al., 1974). The levels of fat in livers of birds that died were usually very high (46 to 83% dry weight) with a mean of 65.2% of lipid content (Nesheim, Ivy and Norvell, 1969). Microscopic examination showed that fat droplets had accumulated in the cytoplasm of the hepatic cells and these globules had apparently fused into one large globule which altered the contour of the cell and displaced the nucleus (Thayer et al., 1973). The association between incidence of haemorrhage and high liver fat content was confirmed by Wolford and Polin (1972), Wolford and Polin (1974) and Garlich et al. (1975).

Changes in the lipid content of the livers of birds with fatty liver syndrome have been reported. Lipid analysis showed that accumulation of fat in the liver of laying hens was largely due to deposition of neutral lipids or triglycerides. The phospholipid content decreased markedly as liver fat increased, but total phospholipids per gram of liver changed relatively little (Ivy and Nesheim, 1973). It was also observed that the linoleic acid content of liver lipid decreased and oleic acid content increased suggesting that deposited lipids originated from biosynthesis rather than from dietary fat.

Although mortality may be associated with the accumulation of fat in the liver, lipid content per se is not the cause of mortality. Death is usually due to a massive haemorrhage. For that reason, Nesheim and Ivy (1970) suggested that the term "Liver haemorrhage syndrome" be used to describe the condition. Hall (1972) also contended that fatty liver syndrome is a misnomer and suggested the term Hepatic (liver) haemorrhage. Blum, Leclercq and Calet (1973) suggested that the term "Haemorrhagic fatty liver" was more appropriate because it refers both to the steatosis and to the loss of blood which may lead to the death of the birds. The name "Fatty liver haemorrhagic syndrome" that also referred to both fat content and occurrence of haemorrhage was used by Wolford and Murphy (1972), Wolford and Polin (1972), Wolford and Polin (1974). Blum, Leclercq and Calet (1973) first used the term "Haemorrhagic liver syndrome" (HLS) to describe the name that had been suggested by Nesheim and Ivy (1970) and this term was also used by Olomu et al., 1975 to describe the condition in laying hens.

ii: Factors affecting the occurrence of haemorrhagic liver syndrome

Numerous factors may be involved in the occurrence of HLS in laying hens. These include genetic factors, environmental influences and the effect of nutrition.

That genetic factors may affect the incidence of HLS was suggested by Nesheim and Ivy (1970). In two populations it was observed that the birds with high liver fat levels showed evidence of liver haemorrhages in 11% of the birds while those with low livers fat levels showed none. Clandinin et al. (1974) reported that haemorrhages were more commonly seen in the livers of one strain of Single Comb White Leghorns than those of other strains and breeds. Garlich et al. (1975) using 20 different varieties of hens and 3 different confinement systems also observed highly significant differences in liver lipid content among the varieties regardless of the confinement system used.

Environmental factors have been shown to exert some effects on the accumulation of fat in the livers and on the incidence of HLS. Nesheim and Ivy (1970) observed mortality from liver haemorrhages during the spring and summer months for several years. High environmental temperatures, together with deficiencies of methionine, choline and vitamin B , have also been shown to cause an increase in the level of liver fat (Wolford, 1971; Schexnailder and Griffith, 1973).

Hartfiel et al. (1970) cited by Ivy and Nesheim (1973) noted that hens kept in cages had higher average liver fat content than hens kept in floor pens. Forced exercise and

consumption of feces caused a reduction in liver fat content of hens housed in cages. Garlich et al. (1975) found that liver lipid content was significantly higher in hens confined two birds per cage than when birds were confined in larger cages at the rate of seven birds per cage or when they were kept in floor pens.

The incidence of nutritional factors on the occurrence of HLS are not clear cut. Couch (1956) noticed that the syndrome is most frequently observed in flocks in which the birds have become heavy because of excessive fattening resulting from feeding a ration with a very high energy content. McDaniel et al. (1959) observed that percent liver fat generally did not appear to be affected by varying the protein, energy or fat level of the diet fed. Nesheim, Ivy and Norvell (1969) also reported that decreasing the calorie-protein ratio by increasing the protein content of the ration or by decreasing the energy content of the ration had no effect on liver fat values. In a subsequent experiment Nesheim and Ivy (1970) observed however that hens fed a low energy diet had lower level of liver fat than those fed higher energy rations.

The use of lipotropic factors in preventing HLS has had variable results. Reed et al. (1968) cited by Wolford and Murphy (1972) showed that inositol was of value in the prevention of HLS. Other workers (Bossard and Combs, 1970;

Leveille and Bray, 1970; Ragland et al., 1970, cited by Wolford and Polin, 1972; and Nesheim and Ivy, 1970) were unable to demonstrate the beneficial effects of the addition of inositol to poultry feeds.

The effects of other lipotropic factors such as choline, vitamin B, vitamin E, and methionine on HLS are obscure. Griffith et al. (1969) reported that supplementing a practical ration for laying hens with choline did not reduce liver fat levels. Nesheim and Ivy (1970), also indicated that choline supplementation had no effect on liver fat levels in hens fed practical rations.

The use of RSM in rations of laying hens has been shown to have an influence on the incidence of HLS. Cardin et al. (1968) observed an increase in mortality in laying hens when RSM in the rations was increased. A similar increase in the incidence of mortality when the percent of RSM was increased in the rations of laying hens was reported by Minetoma (1972). Olomu et al. (1975) also noted that inclusion of RSM in diets of laying hens increased the incidence of HLS. Mortality due to HLS was observed to be 0, 0, 3.1 and 9.4 percent when levels of 0, 5, 7.5 and 10 percent RSM respectively were used in the rations.

There is some evidence that genetic factors may affect resistance to occurrence of HLS when RSM is included in the

rations of laying hens. Jackson (1969) using two strains, Hyline and Hybrid-4 and diets with 0 to 20% of RSM in the rations, found that dietary treatments had no effect on mortality of the Hybrid-4 strain but death rate of the Hyline pullets increased significantly as the level of RSM in the ration increased from 0 to 20%. Confirmation that strain or breed may affect incidence of HLS was obtained by Clandinin et al. (1974). Haemorrhages were more commonly seen in livers of Hyline Leghorns than in White Plymouth Rock, Rhode Island Red or Shaver Leghorns.

EXPERIMENTS AT THE UNIVERISTY OF ALBERTA

Experiments were conducted to study:

Section I: The effect of biotin on the incidence of fatty liver and kidney syndrome in chicks.

Section II: The effect of biotin on the incidence of fatty liver and kidney syndrome in turkey poults.

Section III: The effects of biotin and level of rapeseed meal on the productive performance of laying hens and the incidence of haemorrhagic liver syndrome.

SECTION I

The effect of biotin on the incidence of fatty liver and kidney syndrome in chicks.

Status of the problem

Fatty liver and kidney syndrome has been noted in broilers fed high energy-low protein rations and has resulted in varying levels of mortality. The symptoms of the disorder have been prevented by the addition of biotin to the rations fed. This experiment was conducted in order to determine the effect of supplementation of broiler rations with biotin on incidence of FLKS and on levels of fat present in the livers and kidneys of the birds.

Experimental

Two hundred and forty, day-old, cross-bred (White Mountain x Hubbard White Plymouth Rock), broiler-type chicks were used in this experiment. Four replicates of 10 chicks (5 males and 5 females) were used for each of the six treatments. The experiment was arranged in a 2 x 3 factorial design, involving 2 levels of added biotin (0 and 220 ug/kg) and three levels of protein (22.6, 20.2 and 17.8) in the rations.

The composition of the rations used (Ration 1-6) are shown in Table 1. The rations were formulated to be isocaloric but to contain either a high, medium or low

TABLE 1. Composition of experimental rations for chicks

Ingredients	Protein level					
	High		Medium		Low	
	1	2	3	4	5	6
Ground wheat (13.5% protein) *	75.90	75.50	78.51	78.50	81.10	81.10
Wheat shorts	1.77	1.52	1.77	1.52	1.77	1.52
Stabilized tallow	3.70	3.70	4.10	4.10	4.50	4.50
Meat meal (53% protein)	4.00	4.00	4.00	4.00	4.00	4.00
Herring meal (72% protein)	6.00	6.00	6.00	6.00	6.00	6.00
Isolated soybean meal (91.2% protein)	6.00	6.00	3.00	3.00	---	---
Ground limestone (38% Ca)	1.00	1.00	1.00	1.00	1.00	1.00
Rock phosphate (34% Ca; 18% P)	0.40	0.40	0.40	0.40	0.40	0.40
Iodized salt	0.20	0.20	0.20	0.20	0.20	0.20
Manganese oxide (62% Mn)	0.02	0.02	0.02	0.02	0.02	0.02
Zinc oxide (78% Zn)	0.01	0.01	0.01	0.01	0.01	0.01
Microingredient premix ¹	1.00	1.00	1.00	1.00	1.00	1.00
Biotin premix ²	---	0.25	---	0.25	---	0.25
Calculated analyses						
Metabolizable energy, kcal/kg diet	3120	3129	3131	3131	3133	3133
Protein, %	22.6	22.6	20.2	20.2	17.8	17.8
Lysine, %	1.10	1.10	0.95	0.95	0.79	0.79
Calorie/protein ratio	138	138	155	155	176	176
Calcium, %	1.05	1.05	1.05	1.05	1.04	1.04
Total phosphorous, %	0.71	0.71	0.71	0.71	0.70	0.70
Biotin, µg/kg	107	327	104	324	102	322

¹Supplied the following levels per kg of ration: vitamin A, 6600 I.U.; vitamin D₃, 1320 I.C.U.; vitamin E, 22 I.U.; vitamin K, 1 mg; riboflavin, 4.4 mg; calcium pantothenate, 13 mg; niacin, 22 mg; choline chloride, 187 mg; folic acid, 1 mg; vitamin B₁₂, 10 µg; procain penicillin, 11 mg; DL-methionine, 0.5 g

²Supplied: 220 µg of biotin per kg of ration

protein content. The levels of biotin in the unsupplemented rations were kept low in relation to the chicks requirement by using ingredients that tend to be low in biotin content. The rations were analyzed for crude protein, ether extract (fat) and dry matter content by A.O.A.C. methods (1965).

The initial mean weight of each lot of chicks within the trial were the same. The chicks were wing-banded and brooded in electrically heated batteries with raised screen floors. Continuous lighting was provided. Feed and water were supplied ad libitum.

The chicks were individually weighed at 2 and 4 weeks of age and feed consumption was determined at the end of the trial. When the chicks were weighed they were examined for perosis and dermatitis which are symptoms seen in biotin deficiency. A record of mortality was kept. The trial was terminated when the chicks were 4 weeks old.

At the end of the trial 8 chicks (4 males and 4 females) from each replicate were killed by cervical dislocation. The livers and kidneys were removed, cleaned of adhering blood and fat, and freeze-dried. They were then placed in plastic bags and stored at -15 C until analyzed for fat and protein (Appendix I). The dry matter content of the livers and kidneys were calculated from the fresh and dried weights.

At the conclusion of the experiment, the data were subjected to analysis of variance and significance of differences were assessed by applying Duncan's multiple range test (Steele and Torrie, 1960) at the 0.05 level of probability. Details of the analysis of variance are presented in Appendix II.

Results and Discussion

The effects of protein and biotin levels in the ration on growth, feed conversion and symptoms of biotin deficiency are summarized in Table 2.

Addition of biotin to the ration fed resulted in a significant increase ($P < 0.05$) in average body weight and a decrease in feed required per unit of gain. The increase was greater on the high and medium protein rations but no response was obtained on the low protein ration.

Average body weight and feed conversion were affected by the level of protein in the ration fed. On the rations containing high and medium levels of protein, body weight at 4 weeks of age was significantly greater than on the low protein rations. Feed required per unit of gain was significantly higher on the low protein rations than on the high protein rations.

There was considerable variability in level of

TABLE 2. Effects of protein and biotin levels in rations on performance of chickens.

Ration No.	Protein level	Biotin added $\mu\text{g}/\text{kg}$	Sex	Average body weight g	4 wks	Feed conversion	Mortality %	Deficiency symptoms	
								Perosis %	Dermatitis %
1	High	0	F	596				10	0
			M	625				45	0
			F+M	610 ^a	1.81		7.5	27.5	
2	High	220	F	633				5	0
			M	714				15	0
			F+M	673 ^b	1.70		2.5	10	
			Avg	642 ^a	1.78 ^a		5.0	18.8	
3	Medium	0	F	558				10	0
			M	636				40	0
			F+M	597 ^a	2.00		7.5	25	
4	Medium	220	F	632				15	0
			M	690				25	0
			F+M	661 ^b	1.71		0.0	20	
			Avg	629 ^a	1.85 ^{ab}		3.75	22.5	
5	Low	0	F	520				10	0
			M	540				50	0
			F+M	530 ^c	2.03		2.5	30	
6	Low	220	F	506				5	0
			M	538				30	0
			F+M	522 ^c	1.99		0.0	17.5	
			Avg	526 ^b	2.01 ^b		1.25	23.8	
Combined averages		0		579 ^e	1.95 ^e		5.8	27.5 ^e	
				619 ^f	1.80 ^f		1.7	15.8 ^f	

In the main section of the table and in the combined averages column values with the same superscript or no superscript were not significantly different ($P < 0.05$)

mortality on the different treatments. No significant differences between the groups were observed; however, mortality tended to be lower in the groups fed rations supplemented with biotin.

The birds were examined for incidence of perosis and dermatitis because these are symptoms commonly seen in a biotin deficiency. The chicks fed the biotin deficient rations showed a significantly higher level of perosis than those fed the rations supplemented with biotin; however, biotin was not effective in entirely preventing this disorder. Incidence of perosis was significantly higher among the male birds as compared to the females. No symptoms of dermatitis were seen in this experiment.

The effects of treatments on the composition of livers and kidneys (Table 3) indicated that the level of protein used had no effect on the weight of fresh livers and kidneys, expressed on the basis of body weight. Levels of protein in the ration fed also had no effect on levels of dry matter, fat or protein in liver and kidney tissues of the chicks.

Some significant differences between males and females in the composition of their kidneys were observed. Percentages of dry matter and fat in the kidney tissue of females were slightly higher than those in males. Fresh

TABLE 3. Influence of protein levels, sex, and levels of added biotin on average body weight and composition of livers and kidneys of chicks.

	Treatment Variables.							
	Protein Content of Rations				Sex		Biotin added	
	High	Medium	Low		F	M	0	220 ug/kg
Average body weight, g	642	629	526		574	624	579	619
LIVER								
Fresh weight, g	25.0 ^a	24.9 ^a	18.7 ^b		21.8 ^c	24.0 ^d	24.1	21.7
Dry matter, %	26.2	25.9	25.5		26.2 ^c	25.4 ^d	25.8	25.8
g/100 g body weight, g	3.07	3.17	2.71		3.00	2.97	3.26 ^e	2.70 ^f
Fat, %	12.6	10.4	11.7		10.9	12.1	11.9	11.2
Protein, %	65.6	62.7	70.3		62.4	70.1	67.4	65.0
KIDNEY								
Fresh weight, g	8.2 ^a	8.1 ^a	6.5 ^b		7.1 ^c	8.1 ^d	7.4	7.8
Dry matter, %	21.3	21.4	21.6		21.7 ^c	21.3 ^d	21.5	21.4
g/100 g body weight, g	1.01	1.02	0.94		0.97 ^c	1.01 ^d	1.01	0.97
Fat, %	10.6	11.1	11.0		11.1 ^c	10.7 ^d	11.1	10.7
Protein, %	79.1	78.5	78.2		78.8	78.5	78.4	78.9

For each treatment variable row values with the same superscript or no superscript are not significantly different ($P < 0.05$)

kidney weight (g/100g body weight) was 3% lower in females than in males. No sex difference in kidney protein content was observed.

Addition of biotin to the rations resulted in a decrease in liver size when it was expressed on the basis of body weight. The inclusion of biotin had no effect on dry matter, fat or protein content of the liver. No significant differences in the weight, dry matter, fat or protein content of the kidneys were noted.

In this experiment there was no evidence of FLKS. None of the birds that died showed any symptoms of this disorder.

Summary

The effects of varying the protein and biotin levels of the rations fed on incidence of FLKS and composition of livers and kidneys of the chicks were studied. The results obtained indicated the following:

1. There were no significant differences in level of mortality from the various treatments used. No mortality from FLKS was observed in any of the groups.
2. Addition of biotin to the rations fed resulted in increased body weight and efficiency of feed utilization and reduced incidence of perosis.
A significant decrease in liver size (g/100g body

weight) was noted when biotin was added to the rations but no other changes in liver or kidney composition were observed.

3. Average body weight was significantly higher and feed conversion was lower on the high and medium protein rations than on the low protein ration.

SECTION II

The effect of biotin on the incidence of fatty liver and kidney syndrome in turkey poults.

Status of the problem

The occurrence of FLKS has not been reported in turkey poults, however, since chicks may be affected with the disorder the possibility exists that a similar condition might occur in poults. In chicks the syndrome occurs when high energy-low protein rations are fed and is prevented by the inclusion of biotin in the rations. Since a biotin deficiency may occur in poults fed practical rations this experiment was conducted to assess the occurrence of FLKS in poults and to determine the effects of supplemental biotin on the composition of their livers and kidneys.

Experimental

One hundred and twenty, day old, Broad Breasted Bronze turkey poults of mixed sexes were divided into 12 comparable groups of 10 birds each. Two groups were placed on each of six treatments, involving 3 levels of protein (30.0, 24.8 and 19.7) and 2 levels of added biotin (0 and 550 ug/kg).

The composition of the experimental rations (Basal 1-6) is shown in Table 4. The rations were kept isocaloric by adjusting the level of wheat, stabilized tallow, herring meal and isolated soybean protein in the ration. The poults

TABLE 4. Composition of experimental rations for poult

INGREDIENTS	Protein level					
	High		Medium		Low	
	1	2	3	4	5	6
Ground wheat (13.5% protein)	68.00	68.00	74.10	74.10	80.20	80.20
Wheat shorts	1.77	1.145	1.77	1.145	1.77	1.145
Stabilized tallow	---	---	0.700	0.700	1.40	1.40
Meat meal (53% protein)	8.00	8.00	8.00	8.00	8.00	8.00
Herring meal (72% protein)	10.00	10.00	8.00	8.00	8.00	8.00
Isolated soybean protein(91.2% protein)	10.00	10.00	5.00	5.00	6.00	6.00
Ground limestone (38% Ca)	1.00	1.00	1.00	1.00	---	---
Rock phosphate (35.5% Ca; 18% P)	---	---	0.20	0.20	1.00	1.00
Iodized salt	0.20	0.20	0.20	0.20	0.40	0.40
Manganese oxide (62% Mn)	0.02	0.02	0.02	0.02	0.20	0.20
Zinc oxide (78% Zn)	0.01	0.01	0.01	0.01	0.02	0.02
Microingredient premix ¹	1.00	1.00	0.01	0.01	0.01	0.01
Biotin premix ²	---	---	1.00	1.00	1.00	1.00
	---	0.625	---	0.625	---	0.625
Calculated analysis						
Metabolizable energy, kcal/kg diet	2957	2957	2958	2958	2962	2962
Protein, %	30.0	30.0	24.8	24.8	19.7	19.7
Lysine, %	1.59	1.59	1.23	1.23	0.88	0.88
Calorie/protein ratio	99	99	119	119	150	150
Calcium, %	1.37	1.37	1.37	1.37	1.37	1.37
Total phosphorous, %	0.87	0.87	0.86	0.86	0.85	0.85
Biotin, µg/kg	133	683	122	672	110	660

¹Supplied the following levels per kg of ration: vitamin A, 6600 I.U.; vitamin D₃, 1320 I.C.U.; vitamin E, 22 I.U.; vitamin K, 1 mg; riboflavin, 4.4 mg; calcium pantothenate, 11 mg; niacin, 22 mg; choline, 187 mg; folic acid, 1 mg; vitamin B₁₂, 10 µg; procain penicillin, 11 mg; and DL-methionine, 0.5 g.

²Supplied 550 µg of biotin per kg of ration.

were wing-banded and brooded in electrically heated batteries with raised screen floors. Continuous light was provided. Feed and water were supplied ad libitum.

Individual weights were obtained when the poults were 2 and 4 weeks of age. At the end of the experiment the poults were examined individually for symptoms of biotin deficiency. Records of feed consumption and mortality were kept. The trial was terminated when the poults were four weeks of age.

At the end of the trial the remaining poults were killed by cervical dislocation and individual weight of the poults, livers and kidneys were recorded. The livers and kidneys were cleaned of adhering blood and fat and were then freeze-dried. They were then placed in plastic bags and stored at -15 C until analyzed for protein, fat and ash. The dry matter content of the livers and kidneys was calculated from the fresh and dried weights.

At the conclusion of the experiment the data were statistically analyzed in the same manner as was outlined in Section I. Details of the statistical analysis are presented in Appendix III.

Results and Discussion

The effects of protein and biotin levels in the rations on growth and symptoms of biotin deficiency are summarized in Table 5.

Addition of biotin to the high or medium protein rations resulted in increased body weight at 4 weeks of age. On the low protein ration biotin supplementation had no effect on body weight.

Although the level of mortality was high in this experiment, none of the deaths were caused by FLKS. The addition of biotin was effective in markedly reducing mortality level on the high and medium protein levels but had little effect on the birds fed the low protein rations.

Symptoms of biotin deficiency were observed in poult fed the biotin deficient diets. Perosis, and dermatitis were observed at 4 weeks of age in all poult fed the deficient rations. The dermatitis noted was typical of that seen in a biotin deficiency. Scabby encrustations occurred at the beak angles, and around the eyes. The bottom of the feet were thickened and haemorrhagic cracking was observed on the foot pads.

There was evidence that the level of protein supplied on the medium and low protein rations was insufficient to

TABLE 5. Effects of protein and biotin levels in the ration on growth and symptoms of deficiency of poult.

Protein Biotin content added level $\mu\text{g/kg}$	Average 4 wks body weight g	Mortality %	Perosis %	Dermatitis			White barring %
				Beak %	Eye %	Feet %	
High	0	285	35	53.85	100	100	100
	550	<u>420</u>	<u>10</u>	5.55	---	---	---
	Avg	353 ^a	20.3				
Medium	0	324	50	60.00	100	100	100
	550	<u>370</u>	<u>10</u>	5.27	---	---	100
	Avg	347 ^a	30.0				
Low	0	170	40	58.33	100	100	100
	550	<u>161</u>	<u>35</u>	15.38	---	---	100
	Avg	165 ^b	37.5				

For each treatment variable, column values with the same superscript or no superscript are not significantly different ($P < 0.05$).

meet the poult's requirement. Rate of growth was considerably reduced on the low protein ration and symptoms of a lysine deficiency, characterized by white barring of the primary feathers was observed in all poult's receiving the medium and low protein rations.

The effect of treatment on the composition of livers and kidneys (Table 6) indicated that the levels of protein and biotin used had no effect on fresh liver and kidney weight expressed on the basis of body weight. Liver fat and protein content decreased significantly when the low protein level was fed. No change in dry matter or ash content of the livers of the poult's fed the different diets was observed. The composition of the kidneys was not affected by the treatments used.

Summary

The effects of protein and biotin levels in rations for poult's on the incidence of FLKS and on the composition of their livers and kidneys were studied. The results obtained indicated the following:

1. The general level of mortality in the experiment was high; however, no deaths attributable to FLKS were observed.
2. Addition of biotin to the high and medium protein rations resulted in increased average body weight and reduced level of mortality.

TABLE 6. Effect of protein and biotin levels on the composition of livers and kidneys of poult¹.

	Protein level										
	High			Medium						Average Biotin	
	Biotin	Avg	-	+	Biotin	Avg	-	+	Biotin		Avg
LIVER											
g/100 g body weight,	4.06	3.71	3.88	3.77	3.81	3.79	3.88	4.71	4.30	3.90	4.08
Dry matter, %	25.7	25.0	25.4	24.9	25.2	25.1	28.8	24.5	24.2	24.8	24.9
Fat, %	10.0	10.4	10.2 ^a	9.5	9.8	9.7 ^a	9.2	8.6	8.9 ^b	9.6	9.6
Protein, %	64.0	71.1	67.6 ^a	70.1	64.9	67.5 ^a	62.1	54.5	58.3 ^b	65.4	63.5
Ash, %	5.1	5.0	5.0	5.5	4.9	5.2	4.9	4.8	4.8	5.2	4.7
KIDNEY											
g/100g body weight	1.40	1.17	1.28	1.24	1.08	1.16	1.26	1.27	1.26	1.30	1.17
Dry matter, %	20.8	20.3	20.6	20.5	20.8	20.7	20.1	19.4	19.7	20.5	20.2
Fat, %	8.6	9.5	9.1	9.3	10.1	9.7	8.9	9.4	9.2	8.9	9.7
Protein, %	79.4	76.6	77.9	79.2	76.8	78.0	79.4	78.9	79.2	79.4	77.4
Ash, %	7.2	6.6	6.9	6.6	6.3	6.5	7.1	7.3	7.2	6.9	6.8

¹ Fat, protein and ash expressed as a percentage of dry matter

For each treatment variable, row values with same superscript or no superscript are not significantly different ($P < 0.05$)

3. A very high incidence of perosis and dermatitis was observed in the poult fed the rations without added biotin. Inclusion of biotin resulted in a marked decline in incidence of perosis and complete elimination of symptoms of dermatitis.
4. The composition of the livers and kidneys of poult was not affected by the addition of biotin to the rations fed. A reduction in the fat and protein content of the livers of the poult fed the low protein ration was observed.

SECTION III

The effects of biotin and level of rapeseed meal on the productive performance of laying hens and the incidence of haemorrhagic liver syndrome.

Status of the problem:

High levels of RSM in the rations of laying hens have been observed to increase mortality caused by HLS. Since biotin has been found to be effective in preventing FLKS in broilers it seemed desirable to determine whether this vitamin might have any effect on the occurrence of HLS in layers fed varying levels of RSM. In addition, the effect of biotin supplementation on the composition of the livers and kidneys of laying hens was determined.

Experimental:

Seven hundred and sixty eight Single Comb White Leghorns (Shaver Starcross 288) raised on a commercial type growing ration were used in this experiment. At 22 weeks of age they were leg-banded and placed at random in laying cages (2 birds per 30 x 40 cm cage), in 16 groups of 48 birds each. The birds were fed a commercial-type laying ration until they were 24 weeks of age at which time two groups were placed on each of the experimental rations shown in Table 7.

The dietary treatments involved four different levels

TABLE 7. Composition of experimental rations for laying hens

	Rapeseed meal levels							
	0% RSM	2	3	4	5	6	7	8
Ground wheat (13.5% protein)	78.60	78.60	74.90	74.90	71.20	71.20	67.50	67.50
Soybean meal (48.5% protein)	8.40	8.40	5.80	5.80	3.20	3.20	0.60	0.60
Rapeseed meal (35% protein)	--	--	5.00	5.00	10.00	10.00	15.00	15.00
Stabilized tallow	0.70	0.70	2.00	2.00	3.30	3.30	4.60	4.60
Dehydrated alfalfa	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Wheat shorts	0.31	0.06	0.31	0.06	0.31	0.06	0.31	0.06
Ground limestone (38% Ca)	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Calcium phosphate (18.5% Ca; 20.5% P)	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Iodized salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Micronutrients 1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Trace mineral mix 2	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Biotin premix 3	--	0.25	--	0.25	--	0.25	--	0.25
<u>Calculated analyses</u>								
Metabolizable energy, kcal/kg diet	2706	2706	2706	2706	2708	2708	2708	2708
Protein, %	15.2	15.2	15.3	15.3	15.3	15.3	15.4	15.4
Calcium, %	3.07	3.07	3.10	3.10	3.12	3.12	3.14	3.14
Total phosphorous, %	0.72	0.72	0.74	0.74	0.77	0.77	0.79	0.79
Biotin, ug/kg	102	322	133	353	163	383	194	414

¹Supplied the following levels per kg of ration: vitamin A, 6000 I.U.; vitamin D₃, 900 I.C.U.; vitamin E, 22 I.U.; menadione sodium bisulfate, 1 mg; riboflavin, 3.7 mg; calcium pantothenate, 9.8 mg; niacin, 20 mg; vitamin B₁₂, 6.6 µg; penicillin, 5 mg; folic acid, 1.2 mg; choline chloride, 0.5 g; DL-methionine, 0.5 g; ethoxyquin, 50 mg.

²Supplied the following levels per kg of ration: manganese oxide (62% Mn), 0.025%; zinc oxide (78.5% Zn), 0.01%; copper (25.4% Cu), 0.005%.

³Supplied; 220 µg of biotin per kg of ration.

of Span RSM (0, 5, 10, and 15%) and two levels of added biotin (0 and 220 ug/kg). The rations were kept isocaloric and isonitrogenous by varying the levels of wheat, soybean meal and tallow in the rations as the level of RSM was increased. Feed and water were supplied ad libitum. Each group was fed 700 grams of insoluble grit (size No. 3) per week sprinkled on top of the feed.

During the experimental period of 40 weeks, records were kept on total egg production, egg weight (average weight of eggs laid by each group on one day of each week), Haugh unit values and specific gravity (determined on all eggs laid on one day during each 4-week interval) and feed consumption (measured at 4-week intervals). Body weight was determined at the end of the experiment.

Artificial light was provided to supply 14 hours of light per day during the laying period and the room temperature was maintained above 14 C during the experiment. A record of mortality was kept. All birds that died were sent to the Alberta Veterinary Laboratory for autopsy.

At the end of the experiment 12 birds per group (24 birds per treatment) were fasted for 18 hours and were then sacrificed by severing the jugular vein. The livers from each bird were examined and graded visually to obtain an indication of the amount of fat that they contained. The

livers were placed in grades ranging from a rating of 1 for those of normal dark red appearance to a rating of 5 for those which were very pale and friable, (Fig. 1). In order to determine whether the grading was valid, 3 livers of each grade were pooled and analyzed for dry matter, fat, protein and ash. The results are presented in Appendix IV.

Nine birds from each group were taken at random and the livers and kidneys were removed, cleaned of adhering fat and blood, and weighed. For analysis, the livers and kidneys were pooled into three groups of 3 livers or 3 kidneys, homogenized in a Oster blender, and a portion of the homogenates was freeze-dried for 72 hours to constant weight, and dry matter was calculated. The dried samples were kept in a plastic bag and stored at -15 C until analyzed for fat and protein. At the conclusion of the experiment the data were subjected to analyses of variance and significance of differences were assessed by applying Duncan's Multiple Range Test (Steele and Torrie, 1960) at the 0.05 level of probability. Details of the analysis of variance are shown in Appendix V.

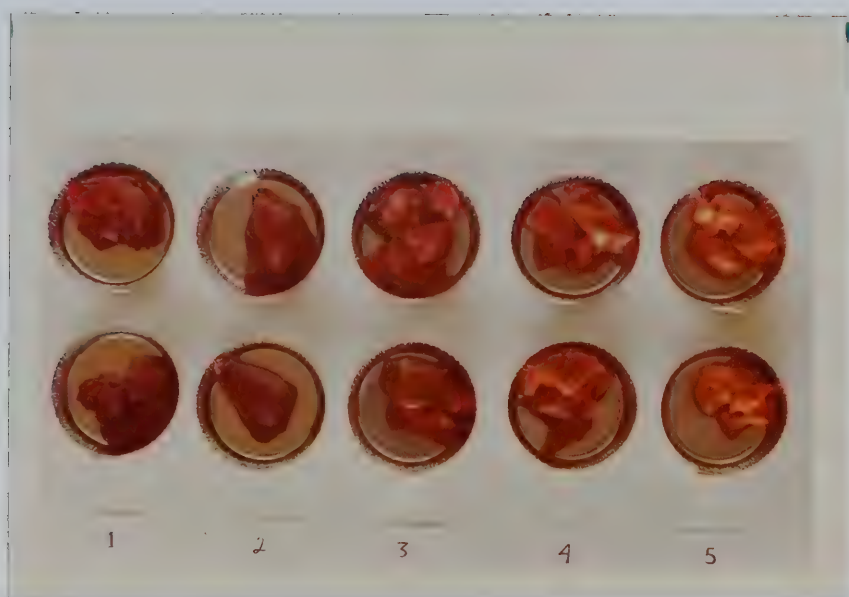
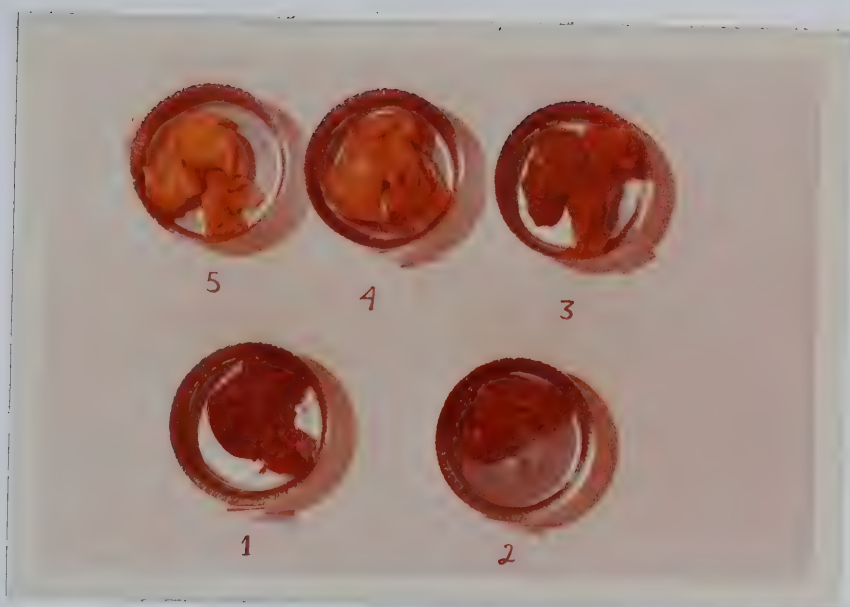


FIGURE 1. "Fatty liver" from laying hens.
(Visual grades used to assess
fatness).

Results and Discussion

A summary of the effects of different levels of RSM and biotin in the rations fed on performance of laying hens is shown in Table 8.

Total mortality rate was considerably higher when 10 or 15% RSM was included in the ration than when 0 or 5% RSM was used, but the differences were not statistically different. No mortality attributable to HLS was observed on rations containing 0 or 5% RSM but HLS accounted for 3.1 and 7.8% mortality on rations containing 10 and 15% of RSM respectively. The increase in mortality from HLS when RSM was increased from 10 to 15% was statistically significant ($P < 0.05$). The inclusion of biotin in the rations (220 ug/kg) had no significant effect on total mortality or mortality caused by HLS.

Rate of production, measured on the basis of the number of birds present at the beginning of the experiment, was significantly reduced in the groups fed rations containing 10 or 15% RSM. When production was calculated on a hen-day basis only, a moderate decrease in production rate occurred as the level of RSM was increased. The treatments used had no significant effect on the amount of feed required to produce a dozen eggs. The addition of biotin to the rations had no effect on rate of production or feed efficiency.

TABLE 8. Effects of levels of rapeseed and biotin supplied on performance of laying hens.

	Rapeseed meal levels					
	0% RSM		5% RSM		10% RSM	
	Biotin +		Biotin -	Biotin +	Biotin -	Biotin +
Total mortality, %	8.3	5.2	5.2	6.2	12.5	11.5
Mortality, (HLS) %	0	0	0	0	1.0	5.2
Average HLS mortality, % ¹	0		0		3.1 ^a	6.3
Production hen-housed, %	75.6	76.1	76.4	75.4	67.3	69.9
Average, hen-housed, %	76.2 ^a		75.9 ^{ab}		68.6 ^b	67.3
Production, hen-day, %	80.7	80.7	79.6	79.3	75.8	76.3
Egg Haugh unit values	83.2	82.6	81.9	80.6	80.9	79.6
Average Haugh unit values	82.9 ^a		81.3 ^{ab}		80.2 ^b	79.1 ^c
Specific gravity	1.08	1.08	1.08	1.08	1.08	1.08
Egg weight, g	55.1	55.3	54.3	54.4	54.6	54.2
Feed per dozen eggs, kg	1.98	1.93	1.89	1.99	2.11	1.97
Initial body weight, kg	1.49	1.46	1.48	1.46	1.48	1.49
Final body weight, kg	1.92	1.90	1.84	1.90	1.90	1.88

¹Row values with same superscript or no superscript are not significantly different (P<0.05)

Biotin supplementation of the rations fed had no effect on egg weight, specific gravity or Haugh unit values of eggs produced. Level of RSM also had no effect on egg weight or specific gravity but average Haugh unit values decreased significantly as the level of RSM was increased. This suggests that high levels of Span RSM in the ration fed may lead to some decrease in interior quality of eggs produced.

Data on the composition of livers and kidneys that were analyzed from each of the experimental groups are presented in Table 9.

The composition of livers and kidneys of laying hens was not affected by the addition of biotin to the rations fed. There were, however, some significant effects on liver composition related to inclusion of RSM in the rations. When RSM was included at levels from 5 to 15%, the dry matter and fat contents of the livers were reduced and the levels of protein were increased. The decrease in dry matter and fat content, as compared to the control ration, was approximately the same on the three levels of RSM. Level of RSM fed apparently had no effect on the dry matter, protein or fat content of kidney tissue.

Summary

Single Comb White Leghorns were fed rations containing 4 levels (0, 5, 10 and 15%) of RSM and 2 levels (0 and 220

TABLE 9. Effects of levels of rapeseed meal and biotin supplied on grades of livers, and composition of livers and kidneys of laying hens.

	Rapeseed meal levels							
	0% RSM		5% RSM		10% RSM		15% RSM	
	Biotin		Biotin		Biotin		Biotin	
	-	+	-	+	-	+	-	+
LIVER								
Grade	3.2	3.2	2.9	3.0	2.8	3.0	3.4	3.3
Fresh weight, g	42.7	38.8	34.2	34.9	36.3	36.8	35.5	36.5
Average, g	40.7 ^a		34.5 ^b		36.5 ^b		36.0 ^b	
g/100 g/body weight, g	2.24	2.12	1.88	2.00	1.98	1.98	1.88	2.07
Dry matter, %	39.6	38.5	33.1	34.1	33.9	35.9	32.8	36.2
Average, %	39.1 ^a		33.6 ^b		34.9 ^b		34.5 ^b	
Protein, %	48.2	48.6	60.3	56.3	59.6	54.4	60.7	53.0
Fat, %	37.7	36.8	28.6	28.7	28.5	31.2	26.6	35.3
KIDNEY								
Fresh weight, g	11.8	11.1	12.2	11.1	13.2	12.8	12.6	12.6
Average, g	11.4 ^a		11.6 ^a		13.0 ^b		12.6 ^b	
g/100 g/body weight, g	0.64	0.61	0.69	0.64	0.73	0.70	0.65	0.62
Dry matter, %	23.3	23.3	23.2	23.4	23.4	23.2	23.4	23.3
Protein, %	72.8	73.7	72.9	72.9	73.6	73.3	72.8	72.5
Fat, %	16.5	16.7	16.5	16.1	16.4	15.8	16.6	17.0

^a Row values with same superscript or no superscript are not significantly different ($P < 0.05$).

ug/kg) of added biotin for an experimental period of 40 weeks. It was observed that:

1. The addition of biotin to laying rations containing levels of RSM ranging from 0 to 15% had no effect on level of mortality or mortality caused by HLS. The use of biotin also had no influence on dry matter, protein or fat content of livers or kidneys. There was also no apparent difference in appearance of livers from hens fed biotin as compared to those receiving unsupplemented rations.
2. No mortality from HLS was observed in rations containing 0 or 5% RSM but HLS accounted for 3.1 and 7.8% mortality when, rations containing 10 and 15% RSM, respectively, were fed.
3. The high levels of RSM (10 and 15%) in the diets significantly reduced the rate of production calculated on a hen-housed production, but when production was calculated on a hen-day basis the decrease that occurred was not significantly different.
4. Egg quality measured in terms of Haugh unit values decreased significantly as the level of RSM in the rations was increased. The treatments used had no effect on specific

gravity, feed per dozen of eggs, or final body weight when laying hens were fed rations containing different levels of RSM.

GENERAL DISCUSSION

The calculated levels of biotin in the unsupplemented rations were sufficiently high to meet the chick's requirement (NAS - NRC, 1971); however, rate of growth was lower and incidence of perosis was higher than on the same rations supplemented with biotin. In the experiment with poult symptoms were observed that were similar to those reported for a biotin deficiency by Patrick et al. (1942) and Robblee and Clandinin (1953; 1970). They included dermatitis, perosis and reduced rate of growth. The performance with chicks and poult suggests that the levels of biotin in the basal rations used were probably borderline with respect to the requirements of the chick and truly deficient in relation to the requirements of the poult.

Despite the fact that a deficiency of biotin was involved in the experiments no symptoms that might be described as FLKS were seen. In addition, supplementing the basal rations with biotin had no effect on the composition of livers and kidneys of either chicks or poult.

The failure to induce FLKS may have been related to the inclusion of fat in the rations fed. Husbands and Laursen-Jones (1969) had reported lower levels of mortality from FLKS on rations containing 4% maize oil or lard. Blair et al. (1973) and Blair, Whitehead and Teague (1975) concluded

that mortality from the disorder was reduced when dietary fat levels were increased. Since broiler rations in Western Canada are usually based on wheat as the principal grain in the ration, stabilized fat is usually added to increase energy content. Under these circumstances losses from FLKS would probably be slight. If, however, shortages of animal fat should develop, or the price of fat should increase so much that it becomes uneconomical to include it in broiler rations, then losses from FLKS might be encountered in the field.

The increased level of mortality from HLS when the level of rapeseed meal used in the ration was 10 or 15% as compared to levels of 5% confirmed previous reports (Jackson, 1969; Clandinin, Robblee and Slinger, 1972; and Olomu et al., 1975). The lower level of mortality observed and the absence of deaths attributable to HLS when 5% rapeseed meal was used, serves to support the current recommendation (Clandinin, Robblee and Slinger, 1972) that the levels of rapeseed meal used in laying diets should not exceed 5% of the ration.

The observation that supplementation with biotin had no influence on total mortality or mortality due to HLS suggests that this vitamin is not of primary importance in development of HLS or prevention of the disorder. The fact that the addition of biotin had no effect on the composition

of the livers or on liver weight (g/100 g body weight) would tend to support the above suggestion since an association seems to exist between incidence of HLS and high liver fat content (Nesheim, Ivy and Norvell, 1969; Nesheim and Ivy, 1970; Wolford and Polin, 1972, 1974; and Garlich et al., 1975).

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APPENDIX I

Procedure for analyses of livers and kidneys

The procedures used for analyses of livers and kidneys have been outlined previously (Olomu et al., 1975). The livers or kidneys were digested with 6N HCL in 600 ml beakers for 1 hour on a reflux apparatus (Gold-Fisch) conventionally used for crude fibre determination. The digesta were made up to volume in 200 ml (turkey kidneys) or 250 ml (chick and laying hen kidneys); 200 ml (turkey livers) or 500 ml (chick and laying hen livers) volumetric flasks with water. For protein determination 20 ml (turkey-chicks) or 10 ml (hens) aliquots were transferred to 800 ml Kjeldahl flasks and protein was determined by A.O.A.C. methods (1965). For fat determination 25 ml (turkeys) or 40 ml (chicks and hens) aliquots were filtered through a No. 42 Whatman filter paper, washed free of acid with hot distilled water, and the residue allowed to air dry. The residue was then extracted for 12 hours with petroleum ether and the fat content calculated according to A.O.A.C. methods (1965).

Appendix II
Analyses of variance-source, degrees of freedom (df) and mean squares-broiler experiment.

Source ¹	df	Mean Squares					
		Final Body wt. g	Feed conversion g/feed g/gain	Liver wt. g/100g body wt.	Kidney wt. g/100g body wt.	Perosis ² %	Fresh Liver g
P	2	.32E+06	.1275	3.71	0.114	195.3	844.56
B	1	94327.0	.1350	14.89	0.796E-01	958.8	280.19
PB	2	34273.0	.33E-01	2.04	0.13E-01	184.7	41.13
R/PB	18	3716.8	.270E-01	1.0797	0.719E-01	330.0	73.89
S	1	0.1479E+06		0.32E-01	0.67E-01	5766.5	235.50
SP	2	9212.8		0.64E-01	0.74E-02	90.9	25.77
SB	1	3096.0		0.58E-02	0.135E-01	745.7	1.50
SBP	2	6569.4		0.10286	0.642E-02	42.2	24.23
SB/PB	18	5682.7		0.3068	0.117E-01	13613.0	14.12

¹P- Protein level; B-Biotin level; R-replicate; S-sex

²Transformed data

Appendix II (cont.)

Source ¹ (df)		Mean Squares						
		Liver			Kidney			
		Dry Matter %	Fat %	Protein %	Dry Matter %	Fat %	Protein %	Mortality %
P	2	8.11	20.04	232.55	3.71	.96	3.46	102.88
B	1	.65E-02	7.16	66.15	.74	2.34	3.21	529.07
PB	2	10.0	9.17	10.62	4.68	.48	.98	44.09
R/PB	18	14.83	11.78	427.97	2.577	.564	2.02	137.17
S	1	30.1	16.83	711.56	10.79	1.31	.74	58.78
SP	2	0.9	18.51	245.49	.477	.13	.18	102.88
SB	1	5.13	25.36	55.87	.80	.15E-03	.20E-03	0.0
SBP	2	1.607	1.778	120.05	1.988	.67E-01	0.850	44.09
SD/PB	18	2.56	12.47	302.98	1.768	.1389	1.987	58.79

¹P-Protein; B-Biotin; R-replicate; S-sex

Appendix III

Analyses of variance-source, degrees of freedom (df)
and mean squares - Poulit experiment.

Source ¹	(df)	Mean Squares					
		Final Body wt. g	Liver wt. g/100g body wt.	Kidney wt. g	Fresh Liver g	Fresh Kidney g	
P	2	4533	0.2918	0.175E-01	103.02	10.978	
B	1	9920	0.903E-01	0.467E-01	30.10	1.290	
PB	2	5176	0.3628	0.141E-01	7.79	0.862	
R+RP+RB +RPB	6	1918	0.1489	0.3317E-01	4.15	0.206	

Source ¹	(df)	Mean Squares									
		Dry Matt. %	Liver Fat %	Liver Protein %	Ash %	Dry Matt. %	Kidney Fat %	Kidney Protein %	Ash %	Mortality %	Hock Disorder
P	2	1.440	1.811	114.01	0.144	1.094	0.408	1.92	.529	167.08	74.8
B	1	0.222E-01	0.619E-02	10.17	0.254	0.250	1.718	11.13	.90E-01	914.73	3806.0
PB	2	0.579	0.236	61.82	0.98E-01	0.245	0.569E-01	1.70	.174	162.64	62.5
R+RP+RB +RPB	6	0.345	0.129	18.88	0.165	0.396	0.328	4.10	.251	122.74	64.5

¹p-Protein levels; B-Biotin levels; R-replicates.

Appendix IV
Liver Grades of Laying Hens

Liver Grade	Fat %	Protein %	Ash %	Dry Matter %
1	13.3 ^a	76.8 ^a	5.5 ^a	28.6 ^a
2	14.9 ^a	74.7 ^a	5.4 ^a	28.8 ^a
3	29.2 ^b	57.7 ^b	4.2 ^b	31.5 ^a
4	46.6 ^c	35.0 ^c	2.6 ^c	46.6 ^b
5	51.9 ^c	24.9 ^d	1.8 ^d	53.9 ^c

Appendix V
Analyses of variance-source, degrees of freedom (df) and mean squares.
Laying hen experiment.

Source*	df	Mean Squares									
		Mortality (Transformed) %	HLS (Transf) %	HHP %	HDP %	Haugh units	Specific Gravity	Egg Weight g	Feed conversion kg feed/doz eggs	Body wt. (Initial)	Body weight (final)
R	3	73.09	232.86	85.62	33.89	10.63	0.149E-05	37.38	0.129E-01	156.8	6055.6
B	1	0.11	34.13	2.56	0.156E-01	3.90	0.249E-06	6.46	0.119E-01	1564.2	1339.6
RB	3	10.22	17.85	2.10	0.100	0.87E-01	0.416E-06	8.53	0.105E-01	239.5	5895.6
DR+DB+DRB	7	31.58	13.29	8.23	8.55	0.535	0.821E-06	15.59	0.182E-01	830.5	1480.2

Mean Squares

Source*	df	Relative organ weight				Liver			
		Liver g/100g body wt.		Kidney		Absolute organ weight		Liver	
		Liver	Kidney	Liver	Kidney	Liver	Kidney	Dry Matt.	Protein
R	3	0.430	0.558E-01	254.13	19.54	70.83	175.60	284.84	
B	1	0.499E-01	0.101E-01	6.25	12.25	20.99	84.42	205.63	
RB	3	0.175	0.149E-01	48.06	2.03	10.20	55.27	34.58	
DR+DB+DRB	7	0.679E-01	0.134E-01	48.31	1.68	0.21	52.46	60.92	

Mean Squares

Source*	df	Liver by Grade Analysis				Kidney			
		Grade		Dry Matt.		Ash		Dry Matt.	
		Fat %	Protein %	Fat %	Protein %	Fat %	Protein %	Fat %	Protein %
R	3	2.38	65.87	211.94	336.29	1.68	0.14E-01	1.26	1.270
B	1	0.208E-01	18.68	20.57	39.06	0.199	0.60E-03	0.138	0.52E-02
RB	3	0.243	4.42	15.22	24.95	0.124	0.10	0.716	0.498
DR+DB+DRB	7	2.223	43.00	120.43	195.84	0.981	0.21	0.812	0.502

* R-rapeseed meal levels; B-biotin levels; D-replicates.

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